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Development and validation of fast UHPLC-MS/MS screening method for 87 NPS and 32 other drugs of abuse in hair and nails: application to real cases

Giulio Mannocchi[1], Annagiulia Di Trana[2], Anastasio Tini[2], Simona Zaami[3], Massimo Gottardi[4], Simona Pichini[5*], Francesco Paolo Busardò[2]

1. University of Camerino, Piazza Cavour 19/f, 62032, Camerino, Italy

2. Department of Excellence of Biomedical Sciences and Public Health, University "Politecnica delle Marche" of Ancona, Via Conca 71, 60124, Ancona, Italy

3. Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Sapienza University, Viale Regina Elena 336 – 00161, Rome, Italy;

4. Comedical S.rL. Via Della Cooperazione 27, 38123 Trento, Italy;

5. National Centre on Addiction and Doping, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy

Corresponding Author:

* Simona Pichini, PhD

National Centre on Addiction and Doping,

Istituto Superiore di Sanità,

Viale Regina Elena 299, 00161 Rome, Italy

simona.pichini@iss.it

Abstract

Interest on keratinized matrices analysis for clinical and forensic purposes has been recently grown due to the wide temporary detection window for psychotropic and toxic substances entrapped after repeated consumption. In this study, the first UHPLC-MS/MS screening method to quantify 119 molecules among most abused classic drugs and new psychoactive substances was developed and fully validated. An amount of 25 mg hair or nails samples, added with the internal standard mixture, were cut and incubated with 500 µL M3® buffer reagent at controlled temperature. After cooling, 1µl supernatant was injected in the chromatographic system equipped with an Oasis HLB column. After the 10 min chromatographic separation through a gradient mobile phase (aqueous ammonium formate, phase A; acetonitrile, phase B), the target compounds were detected in multiple reaction monitoring mode. The method was linear (r^2 always better than 0.99) in a calibration range of 0.01-10 ng compound for mg hair and of 1-200 pg compound per mg nail. Recovery of analytes under investigation was always better than 75% and no significant ion suppression due to matrix effect was observed. Intra-assay and inter-assay precision and accuracy were always better than 15%. The applicability and trueness of the method were examined by analysing real samples of hair and nail from users of psychoactive drugs in recreational contexts. Both classic drugs and new psychoactive substances could be determined as result of repeated use and accumulation in keratin matrices

Keywords: New Psychoactive Substances, UHPLC-MS/MS, hair, nails, alternative matrices

Introduction

Hair and nails are both keratinized biological matrices that can incorporate psychotropic drugs and sequester them for a large time window (e.g. from weeks to months). Hair analysis has long been recognized as an effective tool in toxicological investigations in which proof of repeated drugs consumption in clinical and forensic contexts such as assessment of chronic administration of psychoactive drugs, drug misuse monitoring, workplace drug testing and driving licence re-granting has to be assessed [1]. More recently, nails have been proposed and successfully applied as an alternative or a complement to hair in drug-related intoxications or fatal cases [2-4]. While incorporation of xenobiotics in hair occurs via capillaries, molecules are trapped in nails during their bidirectional growth as principle mechanism. In fact, nails formation occurs from the nail root for the largest part, and from the nail bed during progressive growth for a small part [3]. In addition, drugs could be included in the nail matrix from biological fluids and from occupational exposure [4]. Differently form hair, whose segmental analysis provides time frame of drug consumption considering 1 cm/month growth, nails accounts only for past repeated substance use [2,5].

Besides the consumption of classical illicit drugs, the release of more than 800 new psychotropic molecules belonging to different chemical classes in the illegal market have been reported [6, 7]. These substances have been defined as new psychoactive substances (NPS) by the United Nations Office on Drugs and Crime (UNODC), comprising all those substances not controlled by the 1961 Single Convention on Narcotic Drugs and/or the 1971 Convention on Psychotropic Substances [8].

While consumption trends of NPS are decreasing in the last two years, European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported their use by high-risk drug consumers [7, 9]. In particular, the use of synthetic cathinones and new synthetic opioids together with consumption of new benzodiazepines by opioid and stimulant injectors has

been linked to health and social problems [10-13]. In addition, cannabinoids smoking in party goers and marginalised populations, has been recognized as a threat in a number of European countries [7, 14].

Since the arrival of the NPS on the illicit drug market, consumption patterns have been often characterized by polyconsumption of both classical drugs and NPS. For this reason, without implementing analytical protocols to detect all these substances in clinical and forensic contexts, new health threats for drug consumers may be underestimated [15].

Whereas several methodologies are in place to determine a high number of classic drugs and NPS in conventional biological matrices (eg. blood and urine) [16-23], to our knowledge only few deal with hair specimens and none nails [24, 25].

In this study, a fast and simple method by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to detect and quantify 87 among the most abused NPS and 32 classic illicit drugs and their metabolites in hair and nails has been developed and fully validated. The NPS of interest in our study belong to different chemical classes: 36 synthetic cannabinoids, 22 fentanyl analogues and/or their metabolites, 16 synthetic cathinones, 7 tryptamines, and 6 phenethylamine. Samples from real cases of party goers and psychonauts using different types of psychotropic drugs have been analysed to confirm the suitability of this method.

Materials and Methods

Chemicals and reagents

Working standards of benzoylecgonine, morphine, norfentanyl, amphetamine, dihydrocodeine, codeine, 3,4-methylenedioxyamphetamine (MDA), metamphetamine, 3,4-methylenedioxymetamphetamine (MDMA), 6-O-monoacetylmorphine (6-O-MAM), 3,4-

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methylenedioxy-N-ethylamphetamine (MDEA), norketamine, oxycodone, tramadol, norsufentanyl, zolpidem, lorazepam, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, ketamine, cocaethylene, norbuprenorfina, nordiazepam, flunitrazepam, temazepam, clobazam, phenazepam, methadone, fentanyl, sufentanyl, buprenorphine, Δ9-tetrahydrocannabinol (THC), PX-1, AB FUBINACA, 5-chloro-AB PINACA, PX-2, ADB FUBINACA, AB CHMINACA, MMB 2201, pravadoline, APP FUBINACA, 5-fluoro-ADB, AM-2233, CUMYL-5-fluoro- PINACA, AM-694, JWH-302, CUMYL-PEGACLONE, RCS-4, JWH 251, AM-2201, UR 144, JWH 203, 5-fluoro-NNEI-2, 5-fluoro-AKB48, RCS-8, JWH-018, CP47,497-CB, JWH-016, JWH-098, THJ-018, JWH-081, JWH-122, JWH-019, JWH-007, JWH-210, JWH-147, JWH-398, CB-13, methoxyacetylnorfentanyl, acetylnorfentanyl, butyrylfentanyl-carboxy metabolite, valeryl-fentanyl carboxy metabolite, methoxyacetylfentanyl, furanylnorfentanyl, cis-3-metylnorfentanyl, trans-3-metyl-norfentanyl, butyrylnorfentanyl, cyclopropylnorfentanyl, β-hydroxyfentanyl, alfentanyl, cyclopropylfentanyl, β-hydroxythiofentanyl, furanylethylfentanyl, carfentanyl, butyrylfentanyl, despropionyl-p-fluorofentanyl, 4-anilino-N-phenethyl-piperidine (4-ANPP), phenylacetyl fentanyl, 4-fluoromethcathinone, methcathinone, methylone, mephedrone, buphedrone, ethylone, buthylone, dimthylcathinone, diethylcathinone, 3,4-dimethylmethcathinone, 4-methylethcathinone, ethcathinone, penthedrone, penthylone, methylenedioxypyrovalerone, naphyrone, acetoxy-dimehtyltryptamine, 5-methoxy-a-methyltryptamine, 4-hydroxy-N,N-diehtyltryptamine, 5-methoxy-N,N-monoisopropyltryptamine, 4-acetoxy-N,N-diisopropyltryptamine, 5-methoxy-dipropytryptamine, N,N-diallyl-5-methoxy-tryptamine), 5-(2-aminopropyl)benzofuran (5-APB), 6-(2-aminopropyl)benzofuran (6-APB), 1-(benzofuran-6-yl)-N-methylpropan-2-amine (6-MAPB), 1-(benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB), and deuterated internal standards (ISs; benzoilecgonine-d_3, morphine-d_3, amphetamine-d_6, MDA-d_5, codeine-d_3, metamphetamine-d_5, MDMA-d_5, MDEA-d_5, 6-O-monoacetylmorphine-d_3, cocaine-d_3,

ketamine-d$_4$, cocaethylene-d$_3$, norbuprenorphine-d$_3$, methadone-d$_3$, fentanyl-d$_5$, buprenorphine-d$_4$, THC-d$_3$) were purchased from Cayman Chemical (Ann Arbor, MI, USA) and stored at – 20°C until use.

Working standards of acetylfentanyl, furanylfentanyl, ritalinic acid, MPHP were purchased from LGC, (Queens Road, Teddington, Middlesex, UK) and stored at – 20°C until use.

LC-MS grade water, acetonitrile, methanol and LC grade acetone were obtained from Sigma-Aldrich® (Milano, Italy). Ammonium formate buffer was prepared with ≥ 99% purity ammonium formate salt (Sigma-Aldrich®) dissolved in LC-MS grade water. M3® was purchased from Comedical® s.r.l. (Trento, Italy).

Calibrators and quality control solutions

Methanolic standard solutions of each analyte at 10 µg/mL and working solutions containing all 119 non deuterated standards at 0.1 and 5 µg/mL respectively, were prepared and stored at −20° C.

ISs stock solutions and ISs working solution with benzoilecgonine-d$_3$, morphine-d$_3$, amphetamine-d$_6$, MDA-d$_5$, codeine-d$_3$, metamphetamine-d$_5$, MDMA-d$_5$, MDEA-d$_5$, 6-O-monoacetylmorphine-d$_3$, cocaine-d$_3$, ketamine-d$_4$, cocaethylene-d$_3$, norbuprenorphine-d$_3$, methadone-d$_3$, fentanyl-d$_5$, buprenorphine-d$_4$, THC-d$_3$ were prepared in methanol at 1 µg/mL and 0.5 µg/mL respectively.

Calibrator working solutions were prepared in methanol from the standard solutions (5 calibrators along the working concentration range) at the beginning of every analytical session. Low, medium, and high quality controls (QC) working solutions were daily prepared from the standard stock solution in methanol.

Real samples

Drugs-free hair and nails were gently donated by University personnel involved in this study, signing an informed consent. All samples were prescreened by the below-reported method to assess absence of the analytes under investigations.

Ten hair specimens from authentic positive cases were donated as discarded material by the Institute of Forensic Medicine of Strasbourg, France. Presumptive positive fingernails from nine subjects (2-3 mm of clippings from each of the 10 digits) were obtained from the laboratory storehouse of intoxications and post mortem cases. Hair and nails samples were stored in paper envelopes at ambient temperature until analysis.

Sample treatment

After washing twice with dichloromethane, 25mg hair or nail specimens, added with 5μL ISs working solution, were finely cut and incubated with 500 μL M3® reagent at controlled temperature of 100°C for 60 minutes. Then, samples were cooled at room temperature and 1μL of the supernatant was injected directly into the chromatographic system.

Instrumentation

Analysis was performed through an UPLC Acquity I Class (Waters, Milford, Massachusetts, US) equipped with an Oasis HLB (5 μm 4.6 x 20 mm) (Waters, Milford, Massachusetts, US) column, set at the temperature of 50°C. The chromatographic system was interfaced to a Waters XEVO TQ-S Micro (Waters, Milford, Massachusetts, US) tandem quadrupole mass spectrometer.

The chromatographic run lasted 10 minutes with a gradient mobile phase composed by 12.5 mM ammonium formate pH 9.5 (mobile phase A) and acetonitrile (mobile phase B) at the flow rate 1ml/min. Initial conditions were 75:25 (A:B). Phase A was gradually ramped down from 75 to 0% and phase B gradually ramped up from 25 to 100%.

Mass spectrometric analysis was performed in positive ion multiple reaction monitoring (ES+ MRM) mode. Two transitions for each analyte and at least one transition for deuterated standards were selected. Transitions, relative cone voltage and collision energy are reported in Table 1 for all the analytes under investigations.

Method validation

The method was fully developed and validated in hair and nails in accordance with updated established international criteria [26, 27]. The linearity ranged from limit of quantification (LOQ) of each analyte to 20 ng/mg hair and from LOQ of each analyte-1000 pg/mg nail. Accuracy, precision, selectivity, linearity, sensitivity, and carryover were calculated injecting five different daily replicates of calibration points (five points for each calibration curve, including the limit of quantification as the lowest point) and five replicates of quality control (QC) samples (low QC = 0.05 ng/mg, medium QC = 4 ng/mg and high QC = 15 ng/mg in hair; low QC = 10 pg/mg, medium QC = 200 pg/mg, and high QC = 800 pg/mg in hair) along three subsequent working day. Dilution integrity was tested for over-the-curve samples with a concentration 10 and 50 times higher than the highest calibrators, with a dilution in mobile phase before sample treatment, verifying precision and accuracy to be within 15%.

According to the experimental design proposed by Matuszewski et al. [28], analytical recovery and matrix effect (ME) were determined as following : set 1 was composed of 5 replicates of standard analytes diluted in the mobile phase at low, medium, and high QC concentrations; sets 2 and 3 were composed of 5 hair and nails blank samples fortified with analytes after and before extraction, respectively at the same concentration of replicates of set 1; for each analyte and concentration. ME was calculated by dividing mean peak areas of set 2 by set 1 and recovery was calculated by dividing mean peak areas of set 3 by set 2.

Results

Chromatography and method validation

The method here presented allowed to detect all the target analytes in a 10 minutes run time after a simple one hour pretreatment of specimens. Moreover, the validation parameters obtained for both the keratinized matrices satisfied the established international criteria (Table 2). No additional peaks due to endogenous substances and carryover interfering with analytes and ISs were detected. The method was linear for all analytes under investigation with a determination coefficient (r^2) always better than 0.99. Limits of detection (LODs) ranged from 0.5-6 pg/mg in nails and 0.001-0.006 ng/mg in hair while LOQs ranged between 0.001 and 0.030 ng/mg in hair and 1.0 - 35 pg/mg in nails. Recovery of analytes under investigation was always better than 75% and ion suppression due to matrix effect within 13%. Finally, intra-assay and inter-assay precision and accuracy were always higher than 15%.

Real samples

The present method was applied to screen 10 hair samples obtained from polyconsumers of classic drugs and NPS for which no other data were available, and 9 nails samples obtained from intoxications and post mortem cases of polyconsumers. Target analytes concentration in the positive hair and nails samples are reported in Tables 3 and 4, respectively.

Concerning hair samples, only one sample resulted positive to one substance belonging to NPS (6 APB), while at least three substances were detected in the other samples. NPS belonging to different chemical classes (1 cathinone, 1 tryptamines, 2 synthetic cannabinoids, 3 fentanyl analogues and related metabolites) were detected in 7 hair samples, all of them in association at least to another classic drug, as in case of sample "D" in which 5-MeO-AMT and methadone with its main metabolite EDDP were quantified. One sample was tested positive to 13 substances, showing contemporary consumption of

MDMA, ketamine, cocaine, THC, heroin (6-O-MAM associated to lower concentration of morphine and codeine), methadone and butyryl fentanyl. Also the metabolites norketamine, BZG, EDDP and butyryl norfentanyl were quantified.

All the fentanyl analogues consumers (n=3) presented co-intake of others opiates as methadone and/or tramadol, cocaine and amphetamine-type drugs. Mephedrone was detected in one sample associated with low concentration of methadone, 6-O-MAM, codeine and morphine, ketamine and norketamine and MDMA. Cocaine at a low concentration (0.3 ng/mg) was detected without its metabolite BZG, probably due an occasional consumption of this drug of abuse.

Concerning real nails samples, 5 different NPS belonging to synthetic cathinones and synthetic cannabinoids were detected in association with classic drugs of abuse in 9 samples. Buphedrone was detected in 3 samples together with at least cocaine and BZG, ketamine and norketamine, MDMA, MDA and methamphetamine. Also mephedrone and lorazepam were detected in two of these samples. Mephedrone was detected in two more samples which presented high concentrations of cocaine and its metabolite BZG, ketamine and its metabolite norketamine, MDMA; MDA, methamphetamine and THC. Three different synthetic cannabinoids were quantified in association to at least another classic illicit drug. JWH-081 was the most prevalent synthetic cannabinoid detected, assumed in combination with at least another synthetic cannabinoid (JWH-122 and/or JWH-398) and two classic drugs of abuse (THC, ketamine or cocaine).

Discussion

The importance of keratinized matrices analysis has recently increased in clinical and forensic toxicology due to the stable accumulation of xenobiotics, repeatedly consumed in

the recent past. The analysis of these specimens allows the detection of drugs in a large window, allowing the monitoring of exposure over time and chronic consumption of substances such as drugs of abuse. Although nails and hair analysis do not represent an alternative to traditional matrices analysis, they could be considered complementary in complex toxicological cases. Anyway, while guidelines for hair analysis have been published by scientific organization, such as Society of Hair Testing (SoHT) and Substance Abuse and Mental Health Services Administration (SAMHSA), and official cut-offs for different substances have been proposed, the interpretation of nails analysis results is complicated since information of pharmacokinetics of incorporation of drugs in nails are still ambiguous.

To date, several LC-MS/MS methods to detect a wide range of substances in nails and hair were published. Recently, a comprehensive LC-HRMS method to detect and quantify classical drugs of abuse and NPS in hair was developed and validated by Vincenti et al. [29] The method described is characterized by a fast pretreatment of the sample, in which both the decontamination and extraction of analytes from hair specimens were carried out by pressurized liquid extraction followed by the clean-up of the extracts through dynamic liquid/liquid micro-extraction. Even though the analytical techniques (HPLC interfaced to a single quadruple tandem Orbitrap mass spectrometer) allowed a good sensitivity and specificity, it makes this method hard to be included as a routine analysis in toxicological laboratories, due to the expensive instrumentation and the expertise personnel required.

To the best of our knowledge, an UHPLC-MS/MS method to detect and quantify classic drugs of abuse, NPS and their metabolites in hair and nails specimens have been developed and fully validated for the first time. The analytes under investigation in this study are representative of most important classes of substances abused, taking into account not only the commercial availability of reference standards, but also recent trend of abuse and published scientific literature. [6, 30] The range of linearity for every analyte includes the cut-

off proposed for substances in hair and the concentration expected to be detected in each matrices in acute intoxication or fatal cases, according to values reported in literature.[1, 2, 31] The method was applied to screen real hair and nails samples.

The method here reported allows us not only to screen a broad range of compounds belonging to different chemical classes in a single run of 10 minutes, but also to reach a good analytical sensitivity. The best advantages of this method are represented by the very simple and fast pretreatment and the application in different keratinized matrices.

Conclusion

An ultra-fast, simple and comprehensive UHPLC-MS/MS method for quantifying simultaneously classic illicit drugs and NPS in hair and nails was developed and validated for the first time. Moreover, the current method can be easily expanded to include a greater number of analytes. The use of the M3® reagent allowed us to reduce the time for the pretreatmeant of the samples, making it eligible for high throughput analysis. The application of this method to the screening of small number of real samples allowed us the detection of polydrug consumption, that is the health treat of the moment.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies requiring ethical approval.

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Table 1. Instrumental parameters for analytes under investigation

n.	Analyte	Retention time (min)	Cone Voltage (V)	Quantifier MRM transitions (m/z)	Collision energy (eV)	Qualifier MRM transition (m/z)	Collision energy (eV)
				classic drugs			
1	Benzoylecgonine-d$_3$	0.38	30.00	293.1 > 171.1	20.00	-	-
2	Benzoylecgonine	0.38	30.00	290.1 > 168.1	20.00	290.1 > 105.1	33.00
3	Morphine-d$_3$	0.75	35.00	289 > 61	28.00	-	-
4	Morphine	0.76	35.00	286 > 165.1	40.00	286 > 153	40.00
5	Norfentanyl	0.93	25.00	233.1 > 84.2	20.00	233.1 > 55.3	34.00
6	Amphetamine-d$_6$	1.01	10.00	142.2 > 93.1	16.00	-	-
7	Amphetamine	1.01	15.00	136 > 119.1	8.00	136 > 91.1	15.00
8	Dihydrocodeine	1.03	35.00	302.1 > 199.1	34.00	302.1 > 201.1	30.00
9	Codeine	1.18	30.00	300.1 > 215.1	25.00	300.1 > 199.2	27.00
10	3,4-Methylenedioxyamphetamine (MDA)	1.20	20.00	180 > 133.1	18.00	180 > 163.1	10.00
11	Metamphetamine	1.21	20.00	150.1 > 91.1	12.00	150.1 > 119.1	10.00
12	MDA-d$_5$	1.23	20.00	184.71 > 137.2	18.00	184.71 > 109.7	10.00
13	Codeine-d$_3$	1.26	40.00	303 >215.1	25.00	303 > 199.1	30.00
14	Metamphetamine-d$_5$	1.29	20.00	154.8 > 91.8	12.00	154.8 > 119.1	10.00
15	3,4-Methylenedioxymetamphetamine- d$_5$ (MDMA-d$_5$)	1.29	20.00	199.1 > 1165.1	12.00	199.1 > 135.25	20.00
16	MDMA	1.29	20.00	194.1 > 163	14.00	194.1 > 133.1	20.00
17	6-O-Monoacetylmorphine (6-O-MAM)	1.46	30.00	328.1 > 165.1	40.00	328.1 > 181.2	40.00
18	3,4-Methylenedioxy-N-ethylamphetamine- d$_5$ (MDEA-d$_5$)	1.51	20.00	213.1 > 163.1	14.00	213.1 > 105.1	26.00
19	MDEA	1.55	20.00	208.1 > 163.1	14.00	208.1 > 135.1	14.00
20	Norketamine	1.90	20.00	224.1 > 207.1	10.00	224.1 > 125	25.00
21	Oxycodone	1.99	25.00	316.1 > 241.2	30.00	316.1 > 256.1	30.00
22	Tramadol	2.02	25.00	264.1 > 58.1	15.00	-	-
23	Norsufentanyl	2.02	25.00	277 > 128.1	15.00	277 > 96	25.00
24	6-O-MAM-d$_3$	2.09	30.00	331 > 61.1	30.00	-	-
25	Zolpidem	2.13	45.00	308.1 > 235.2	34.00	208.1 > 263.1	28.00
26	Lorazepam	2.21	30.00	312.10 > 229.0	30.00	321.0 > 275.0	20.00
27	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	2.28	45.00	278.2 > 234.2	26.00	278.2 > 186.2	35.00
28	Cocaine	2.57	30.00	304.20 > 182.26	20.00	304.2 > 82.3	30.00

29	Cocaine-d$_3$	2.59	30.00	307.0 > 184.7	20.00	307.0 > 84.8	30.00
30	Ketamine-d$_4$	2.61	20.00	242.2 > 129.1	25.00	242.2 > 211.1	15.00
31	Ketamine	2.62	20.00	238.2 > 125.1	25.00	238.2 > 220.2	15.00
32	Cocaethylene	2.89	30.00	318.1 > 196.1	20.00	318.1 > 82.1	30.00
33	Cocaethylene-d$_3$	2.93	30.00	321.1 > 199.1	20.00	321.1 > 85	30.00
34	Norbuprenorphine-d$_3$	2.95	55.00	417.2 > 100.8	40.00	417.2 > 56.8	40.00
35	Norbuprenorfine	2.95	55.00	414.2 > 101.3	40.00	414.2 > 83.2	55.00
36	Nordiazepam	2.97	40.00	271.1 > 140	27.00	271,1 > 165,1	27.00
37	Flunitrazepam	3.02	52.00	314.1 > 239.1	36.00	314,1 > 268,1	26.00
38	Temazepam	3.04	34.00	301.0 >177.0	20.00	301.0 > 255	38.00
39	Clobazam	3.04	38.00	301,1 > 224	36.00	301,1 > 259	22.00
40	Phenazepam	3.08	25.00	350.7 > 104.7	45.00	350.7 > 206.0	35.00
41	Methadone	3.27	30.00	310.3 > 265.2	14.00	310.3 > 105.1	32.00
42	Methadone-d$_3$	3.30	38.00	313.2 > 105.1	28.00	-	-
43	Fentanyl	3.87	35.00	337.2 > 105.2	38.00	337.2 > 188.2	30.00
44	Fentanyl-d$_5$	3.89	35.00	342.2 > 105.2	38.00	342.2 > 188.2	30.00
45	Sufentanyl	4.41	16.00	387.2 > 238.1	38.00	387.2 > 111	18.00
46	Buprenorphine-d$_4$	5.65	55.00	472.2 > 59.2	80.00	-	-
47	Buprenorphine	5.65	55.00	468.2 > 55.2	80.00	468.2 > 84.2	40
48	Δ9-tetrahydrocannabinol-d$_3$ (THC-d$_3$)	5.79	45.00	318 > 123	34.00	-	-
49	THC	6.19	45.00	315.2 > 193.1	34.00	315.2 > 123	22.00
	synthetic cannabinoids						
50	PX-1	2.80	36.00	396.30 > 144.00	44.00	379.1 > 134.8	24.00
51	AB FUBINACA	2.83	36.00	369.30 > 109.00	40.00	369.30 > 253.00	24.00
52	5 CL AB PINACA	2.94	36.00	366 > 249	24.00	366 > 145	44.00
53	PX-2	2.97	26.00	397.30 > 145.00	46.00	397.3 > 233.0	22.00
54	ADB Fubinaca	3.15	25.00	383.2 > 109.0	42.00	383.20 > 253.00	25.00
55	AB CHMINACA	3.46	38	357.4 > 241.2	28.00	357.4 > 145	46.00
56	MMB 2201	3.52	34.00	363.3 > 231.9	12.00	363.3 > 143.9	38.00
57	pravadoline	3.77	45.00	379.1 > 113.9	32.00	379.1 > 134.8	24.00
58	APP FUBINACA	3.9	20.00	417.3 > 109	40.00	417.3 > 253	24.00
59	5-F ADB	4.1	45.00	378.30 > 105.00	24.00	378.30 > 318.00	14.00
60	AM-2233	4.75	45.00	459.0 > 111.9	22.00	459.0 > 97.8	34.00
61	CUMYL 5F PINACA	4.77	32.00	368.3 > 250	10.00	368.3 > 233	18.00
62	AM-694	4.99	45.00	436.0 > 202.7	40.00	436.0 > 230.70	28.00
63	JWH 302	5.16	45.00	336.10 > 121.10	22.00	322.0 > 134.80	26.00
64	CUMYL PEGACLONE	5.26	30.00	373.3 > 255	10.00	373.3 > 119	24.00

65	RCS-4	5.33	45.00	322.0 > 106.80	40.00	322.0 > 134.80	24.00
66	JWH 251	5.51	45.00	319.8 > 105.9	22.00	319.8 > 214.20	15.00
67	AM-2201	5.59	45.00	360.20 > 126.90	40.00	360.20 > 154.90	28.00
68	UR 144	5.68	18.00	312.20 > 55.0	36.00	312.20 > 125.00	22.00
69	JWH 203	5.69	45.00	340.4 > 124.8	28.00	340.4 > 187.8	20.00
70	5f NNEI-2	5.76	22.00	375.3 > 232	20.00	375.3 > 144	42.00
71	5F-AKB48	5.93	35.00	384.0 > 106.9	45.00	384.0 > 134.90	25.00
72	RCS-8	6.13	45.00	376.10 > 90.85	40.00	376.10 > 120.83	26.00
73	JWH 018	6.27	45.00	342.10>127.00	25.00	342.10 >155.00	34.00
74	CP47, 497-C8	6.32	45.00	386.70 > 104.80	22.00	386.70 > 120.80	24.00
75	JWH 016	6.33	45.00	341.70 > 127.10	44.00	341.70 > 155.10	24.00
76	JWH 098	6.33	45.00	385.80 > 157.20	42.00	385.80 > 185.10	26.00
77	THJ 018	6.36	25.00	377.2 > 248.9	16.00	377.2 > 212.9	24.00
78	JWH 081	6.38	45.00	371.80 > 157.09	40.00	371.80 > 185.08	26.00
79	JWH 122	6.48	45.00	356.10 > 140.90	40.00	356.10 > 168.80	26.00
80	JWH 019	6.52	45.00	356.10 > 255.07	26.00	356.10 > 228.10	26.00
81	JWH 007	6.56	45.00	355.80 > 127.09	48.00	355.80 > 155.09	26.00
82	JWH 210	6.69	45.00	369.80 > 183.10	26.00	369.80 > 214.20	24.00
83	JWH 147	6.70	45.00	382.10 > 127.09	48.00	382.10 > 155.06	22.00
84	JWH 398	6.90	45.00	376.06 > 161.07	48.00	376.06 > 189.06	26.00
85	CB-13	7.71	45.00	369.20 > 155.08	26.00	369.20 > 170.80	28.00
	fentanyl analogues						
86	Methoxyacetyl norfentanyl	0.50	15.00	249.1 > 84.1	14.00	246.1 > 55	38.00
87	Acetyl Norfentanyl-d5	0.58	25.00	224.2 > 84	18.00	-	-
88	Acetyl Norfentanyl	0.59	25.00	219.2 > 84.05	18.00	219.2 > 55.2	36.00
89	Butyryl Fentanyl Carboxy metabolite	0.76	25.00	381.2 > 105	42.00	381.2 > 188.1	30.00
90	Valeryl Fentanyl Carboxy metabolite	0.87	40.00	395.3 > 105.25	44.00	395.3 > 188.15	26.00
91	Methoxyacetylfentanyl	1.1	30.00	353.3 > 188	20.00	249.1 > 84.1	18.00
92	Furanyl Norfentanyl	1.15	16.00	271 >84.2	18.00	271 > 55.1	38.00
93	Cis-3-Metyl Norfentanyl	1.15	25.00	247 > 98	16.00	247 > 69.2	30.00
94	Trans-3-Metyl Norfentanyl	1.15	25.00	247 > 98.1	16.00	247 > 69.1	30.00
95	Butyryl Norfentanyl	1.26	25.00	247.1 > 84.15	20.00	247.1 > 55.3	36.00
96	Cyclopropyl Norfentanyl	1.33	25.00	245.2 > 177.1	18.00	245.2 > 84.1	20.00
97	β-hydroxyfentanyl	2.5	25.00	389.2 > 238	16.00	389.2 > 111	38.00
98	Alfentanyl	2.6	24.00	417.1 > 197.05	26.00	417.1 > 268.1	16.00
99	Cyclopropylfentanyl	3.06	25.00	349.2 > 105	30.00	349.2 > 188.1	25.00
100	β-hydroxythiofentanyl	3.14	25.00	359.2 > 192	22.00	359.2 > 111	38.00

101	**Furanylethyl Fentanyl**	3.31	25.00	327.2 > 95.1	34.00	327.2 > 178.1	16.00
102	**Acetyl fentanyl**	3.33	25.00	322.2 > 105	36.00	322.2 > 188	20.00
103	**Furanyl Fentanyl**	3.5	30.00	375.1 > 188	20.00	375.1 > 105	25.00
104	**Carfentanyl**	3.93	22.00	395.2 > 113	32.00	395.2 > 335	16.00
105	**Butyryl Fentanyl**	4.22	30.00	351.2 > 105	40.00	351.2 > 188.1	22.00
106	**Despropionyl-para-fluorofentanyl**	4.52	15.00	299.1 > 105.1	16.00	299.1 > 188.1	38.00
107	**4-anilino-N-phenethyl-piperidine (4-ANPP)**	4.55	22.00	281.2 > 105	22.00	281.2 > 188	14.00
108	**Phenyl Acetyl Fentanyl**	4.96	46.00	399.3 > 188.05	24.00	399.3 > 105.05	44.00
	synthetic cathinones						
109	**4-fluoromethcathinone**	0.83	35.00	205.00 > 102.80	28.00	205.00 > 148.70	26.00
110	**Methcathinone**	0.97	30.00	163.90 > 104.80	22.00	163.9 > 130.7	20.00
111	**Methylone**	1.15	30.00	208.1 > 159.9	16.00	208.1 > 131.9	26.00
112	**Mephedrone**	1.44	30.00	178.01 > 145	18.00	178.01 > 119	22.00
113	**Buphedrone**	1.45	30.00	178 > 130.3	26.00	178 > 91	32.00
114	**Ethylone**	1.63	30.00	222 > 174	20.00	222 > 146	26.00
115	**Buthylone**	1.63	25.00	222 > 173.9	20.00	222 > 145.9	26.00
116	**Dimethylcathinone**	1.80	30.00	177.70 > 72.10	22.00	177.7 > 105.30	20.00
117	**Diethylcathinone**	1.84	25.00	206.30 > 100.00	22.00	206.30 > 105.00	20.00
118	**3,4- dimethylmethcathinone**	1.85	30.00	192.00 > 143.90	28.00	192.00 > 158.80	22.00
119	**4-methylethcathinone**	1.89	35.00	192.00 > 145.30	18.00	192.00 > 91.00	34.00
120	**Ethcathinone**	1.94	30.00	177.7 > 72	16.00	177.7 > 105.20	22.00
121	**Penthedrone**	2.09	35.00	192.10 > 90.90	20.00	192.10 > 131.70	20.00
122	**Penthylone**	2.15	35.00	236.10 > 174.90	22.00	236.10 > 187.80	18.00
123	**Methylenedioxypyrovalerone (MDPV)**	3.42	30.00	276.1 > 126	26.00	276.1 > 134.8	24.00
124	**Naphyrone**	4.93	45.00	282.10 > 126.2	36.00	282.10 > 140.9	26.00
	Tryptamines						
125	**Acetyl-o-dimethyltryptamine (ACO DMT)**	1.33	28.00	247 > 58.1	24.00	247 > 160	14.00
126	**5-methoxy-α-methyltryptamine (5-MeO-AMT)**	1.33	22.00	205.1 > 147	20.00	205.1 > 173	22.00
127	**4-hydroxy-diethyltryptamine (4 OH DET)**	1.6	16.00	233.1 > 86.1	18.00	233.1 > 160	14.00
128	**5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MIPT)**	1.8	10.00	247.1 > 86	14.00	247.1 > 174	16.00
129	**4-acetoxy-diisopropyltryptamine (4-AcO-DIPT)**	1.99	15.00	303.1 > 114	18.00	303.1 > 160	28.00
130	**5-methoxy-dipropyltryptamine (5-MeO-DPT)**	2.92	14.00	275.2 > 174	16.00	275.2 > 114	14.00
131	**5-methoxy-diallyltryptamine (5-MeO-DALT)**	3.78	24.00	271.2 > 110	14.00	271.2 > 174	18.00
	Phenylethylamines						

132	Ritalinic Acid	0.63	20.00	220.1 > 84.1	20.00	220.1 > 56	46.00
133	5-(2-aminopropyl)benzofuran (5-APB)	1.74	26.0	176.2 > 91	28.00	176.2 > 77	38.00
134	6-(2-aminopropyl)benzofuran (6-APB)	1.75	22.00	176.2 > 91	26.00	176.2 > 77	40.00
135	1-(benzofuran-6-yl)-N-methylpropan-2-amine (6-MAPB)	1.83	22.00	190.15 > 159	10.00	190.15 > 131	18.00
136	1-(benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB)	2.11	24.00	204.15 > 131	20.00	204.15 > 91	30.00
137	4'-methyl-α-pyrrolidinohexiophenone (MPHP)	4.20	10.00	260.2 > 105	22.00	260.2 > 189	16.00

Table 2. Validation parameters for analytes under investigation in hair and nails. Low, medium and high quality control samples (QC) for hair contained at 0.05 ng/mg, 4 ng/mg and 15 ng/mg, respectively. Low, medium and high QC samples for nails contained at 10 pg/mg, 200 pg/mg and 800 pg/mg, respectively. Analytical recovery and matrix effect are displayed as mean value of low, medium and high QC values.

Analyte	Matrix	Determination coefficient (r2)	LOD (ng/mg in hair; pg/mg in nails)	LOQ (ng/mg in hair; pg/mg in nails)	Accuracy (%err) low QC	mid QC	high QC	Intra-assay precision (%CV) low QC	mid QC	high QC	Inter-assay precision (%CV) low QC	mid QC	high QC	Matrix effect (%)	Recovery (%)
classic illicit drugs
Benzoylecgonine	hair	0.9998	0.006	0.015	16.6	5.2	2.2	10.9	5.4	4.2	16.3	3.0	6.2	90.2	87.0
	nails	0.9997	6	12	15.0	10.2	4.0	12.0	8.3	2.9	15.9	10.1	4.8	95.4	79.7
Morphine	hair	0.9989	0.008	0.005	14.3	6.1	3.1	10.6	5.6	4.6	16.5	2.9	6.2	89.6	91.6
	nails	0.9993	0.8	4	13.5	11.1	3.8	12.2	8.2	3.2	16.0	9.8	4.8	91.2	95.7
Norfentanyl	hair	0.9986	0.005	0.002	17.1	4.8	2.1	11.2	5.3	4.3	15.9	2.8	6.2	88.9	94.2
	nails	0.9995	0.5	1	12.6	9.2	4.3	12.0	8.7	3.2	16.0	9.7	4.7	91.4	96.1
Amphetamine	hair	0.9972	0.05	0.030	13.2	4.8	3.5	10.4	5.6	3.8	15.9	2.9	6.5	90.2	88.1
	nails	0.9984	5	35	12.8	9.2	4.0	11.8	7.9	2.7	16.3	9.5	4.6	92.1	97.7
Dihydrocodeine	hair	0.9993	0.008	0.005	17.4	5.6	1.9	10.6	5.1	4.0	16.3	3.0	6.3	89.3	94.3
	nails	0.9982	0.8	4	16.2	8.3	2.5	12.2	8.5	2.9	15.8	9.6	4.9	94.2	95.4
Codeine	hair	0.9983	0.008	0.005	17.5	5.3	1.9	10.8	5.5	4.1	16.2	3.3	6.0	89.4	98.6
	nails	0.9988	0.8	4	16.8	6.1	2.2	11.9	8.2	2.8	15.7	10.2	5.0	95.1	96.9
MDA	hair	0.9972	0.005	0.030	16.2	7.4	3.1	11.1	5.4	4.2	16.3	3.0	6.1	90.3	95.0
	nails	0.9989	5	35	14.7	5.3	3.6	12.1	8.3	3.2	15.9	10.2	4.8	91.5	97.0
Metamphetamine	hair	0.9972	0.005	0.030	16.3	7.8	2.8	10.5	5.7	4.5	16.4	3.1	6.4	91.2	87.4
	nails	0.9982	5	35	15.8	6.1	2.2	12.3	8.6	3.1	16.1	10.3	4.7	91.8	96.1
MDMA	hair	0.9989	0.005	0.030	16.5	7.7	2.5	11.3	5.0	4.6	16.5	3.0	6.0	90.7	85
	nails	0.9994	5	35	15.8	7.1	2.2	12.0	8.0	3.1	15.9	9.9	4.6	92.5	91
6-O-MAM	hair	0.9998	0.001	0.005	15.8	6.8	1.8	11.2	5.3	3.8	16.3	2.8	6.2	89.9	86.9
	nails	0.9982	0.8	4	12.5	7.2	2.3	12.1	8.2	2.9	15.7	10.1	4.6	91.1	96.4
MDEA	hair	0.9984	0.005	0.030	16.7	7.1	2.3	10.9	5.3	4.2	16.0	2.9	6.0	90.6	81.7
	nails	0.9997	5	35	14.2	6.8	2.2	11.7	8.1	2.7	15.6	10.1	5.0	90.9	89.6
Norketamine	hair	0.9993	0.005	0.012	15.8	4.3	2.1	10.4	5.6	4.3	16.0	3.0	6.1	91.2	78.9

	nails	0.9983	5	10	16.1	4.8	1.8	12.2	8.5	3.2	16.1	10.2	4.8	92.3	85
Oxycodone	hair	0.9971	0.005	0.015	15.8	6.8	2.2	10.6	5.5	4.4	15.9	3.1	5.9	94.0	90
	nails	0.9998	5	10	16.2	7.3	2.1	11.5	8.3	2.9	16.1	10.0	4.8	95.8	94.4
Tramadol	hair	0.9981	0.005	0.001	16.1	5.2	1.9	11.1	5.2	4.1	16.3	3.4	6.2	89.3	89.7
	nails	0.9992	0.5	1	15.9	5.8	2.1	12.3	8.5	2.8	16.0	10.1	4.9	92.1	91.4
Norsufentanyl	hair	0.9971	0.005	0.002	17.1	4.8	2.1	10.7	5.4	4.7	15.9	2.9	6.1	90.2	88.4
	nails	0.9996	0.5	1	12.6	9.2	4.3	12.0	8.1	2.9	15.9	10.1	4.7	91.5	89.1
Zolpidem	hair	0.9989	0.005	0.010	14.5	5.6	2.4	10.9	5.7	4.2	16.2	2.9	6.2	89.7	75.7
	nails	0.9995	5	10	12.1	6.1	2.2	11.8	8.4	2.7	15.8	9.9	4.7	91.2	79.0
Lorazepam	hair	0.9991	0.005	0.010	16.5	6.1	1.8	10.8	5.4	4.4	16.1	2.8	6.3	91.5	86.7
	nails	0.9997	5	10	15.8	6.5	2.0	12.1	8.1	3.1	16.2	10.2	4.9	95.3	89.0
EDDP	hair	0.9974	0.001	0.020	15.8	6.8	1.8	11.2	5.5	4.5	16.2	3.0	6.0	90.7	87.5
	nails	0.9982	0.8	15	12.5	7.2	2.3	12.3	8.2	3.2	15.9	10.1	4.9	90.9	91.4
Cocaine	hair	0.9998	0.006	0.015	16.6	5.2	2.2	10.9	5.4	4,2	16.2	3.1	6.3	85.7	90.0
	nails	0.9998	6	12	15.0	10.2	4.0	12.0	8.3	2.9	15.7	9.8	4.8	92.0	93.7
Ketamine	hair	0.9968	0.005	0.012	15.8	4.8	2.1	10.6	5.6	3.8	15.9	2.9	6.1	89.1.	85.4
	nails	0.9978	5	10	16.1	5.0	2.2	11.9	8.3	3.1	15.8	10.2	5.0	93.4	91.6
Cocaethylene	hair	0.9971	0.006	0.015	16.6	5.2	2.2	10.9	5.4	4,2	16.4	3.0	6.2	88.7	81.7
	nails	0.9983	6	12	15.0	10.2	4.0	12.0	8.3	2.9	16.1	10.1	4.9	91.5	93.1
Norbuprenorfine	hair	0.9984	0.005	0.030	16.8	4.8	2.4	11.2	5.6	4.1	16.0	2.9	6.3	92.3	78.1
	nails	0.9982	5	20	15.0	7.2	2.1	12.1	8.4	2.8	15.9	9.9	4.8	95.6	82.6
Nordiazepam	hair	0.9965	0.005	0.010	16.5	6.1	1.8	10.8	5.7	4.2	15.9	3.0	5.9	90.6	76.9
	nails	0.9987	5	10	15.8	6.5	2.0	12.3	8.6	2.7	16.1	9.9	4.8	91.2	88.3
Flunitrazepam	hair	0.9983	0.005	0.010	16.5	6.1	1.8	10.7	5.3	4.3	16.0	3.1	5.9	89.7	78.0
	nails	0.9995	5	10	15.8	6.5	2.0	11.7	8.3	3.2	15.8	10.1	5.0	92.3	87.5
Temazepam	hair	0.9998	0.005	0.010	15.8	6.6	2.2	10.5	5.3	4.5	16.1	3.0	6.0	91.2	89.6
	nails	0.9998	5	10	16.2	10.1	3.1	12.0	8.1	3.2	15.8	10.2	4.8	93.7	85.0
Clobazam	hair	0.9982	0.005	0.010	16.7	7.1	2.3	10.8	5.1	4.1	16.3	2.9	6.1	90.8	85.4
	nails	0.9984	5	10	14.3	6.8	2.1	11.9	8.4	2.9	15.9	10.1	4.9	95.6	87.9
Phenazepam	hair	0.9974	0.005	0.010	15.8	6.2	2.2	11.2	5.6	4.3	16.5	2.8	6.2	89.9	78.9
	nails	0.9987	5	10	16.3	8.5	2.1	11.8	8.2	3.0	15.7	9.9	5.0	91.2	93.9
Methadone	hair	0.9998	0.001	0.020	15.8	6.8	1.8	11.3	5.4	4.2	16.5	3.0	6.0	95.4	83.8

Compound	Matrix														
	nails	0.9996	0.8	15	12.5	7.2	2.3	12.2	8.4	2.8	16.0	9.8	4.8	95.7	79.5
Fentanyl	hair	0.9989	0.001	0.002	17.1	4.8	2.1	10.9	5.5	4.1	16.2	2.9	5.9	92.3	84.0
	nails	0.9996	0.5	1	12.6	9.2	4.3	12.1	8.0	2.9	15.9	10.1	4.9	95.3	91.3
Sufentanyl	hair	0.9982	0.001	0.002	17.1	4.8	2.1	10.7	5.5	4.0	16.3	3.1	6.1	93.7	86.4
	nails	0.9988	0.5	1	12.6	9.2	4.3	12.0	8.1	3.1	15.8	10.2	5.0	96.1	91.3
Buprenorphine	hair	0.9989	0.005	0.030	16.8	4.8	2.4	11.2	5.7	4.2	16.1	3.0	6.2	89.3	78.7
	nails	0.9993	5	20	15.0	7.2	2.1	12.3	8.2	3.1	16.2	9.9	4.9	91.2	81.3
THC	hair	0.9993	0.002	0.025	14.3	5.4	1.8	10.9	5.3	4.5	16.0	2.9	6.2	90.2	83.1
	nails	0.9987	2	20	11.2	6.9	1.6	11.8	8.3	2.8	15.7	9.8	4.8	92.3	88.4
synthetic cannabinoids															
PX-1	hair	0.9971	0.005	0.025	13.4	10.7	4.5	15.4	14	6.4	15.7	12.8	6.9	87.6	89.0
	nails	0.9981	5	20	14.0	9.1	5.0	13.0	12.7	4.8	16.0	12.0	6.6	90.1	92.1
AB FUBINACA	hair	0.9984	0.005	0.025	12.5	10.5	4.3	15.6	13.6	6.4	15.6	12.8	6.9	90.1	91.2
	nails	0.9991	5	20	12.8	9.4	4.8	13.3	12.9	5.0	15.8	11.5	6.5	94.3	92.2
5 CL AB PINACA	hair	0.9981	0.005	0.025	13.6	11.1	4.0	15.4	13.8	6.5	16.0	12.5	6.7	88.5	88.6
	nails	0.9986	5	20	13.8	10.2	4.8	13.5	12.5	5.0	15.6	11.9	6.5	89.4	95.4
PX-2	hair	0.9973	0.005	0.025	13.4	10.7	4.5	15.2	14.2	6.6	15.7	12.6	6.7	91.0	86.5
	nails	0.9987	5	20	14.0	9.1	5.0	13.3	13.0	4.8	15.9	12.2	6.9	92.3	94.5
ADB Fubinaca	hair	0.9989	0.005	0.025	13.7	9.8	3.8	15.7	14.0	6.3	15.5	12.6	6.7	89.7	75.7
	nails	0.9991	5	20	14.5	9.1	4.0	13.1	12.6	4.6	16.2	12.2	6.9	90.1	81.3
AB CHMINACA	hair	0.9974	0.005	0.025	13.2	10.8	4.3	15.5	13.8	6.4	15.9	13.0	6.5	91.2	89.6
	nails	0.9977	5	20	13.5	10.1	5.0	12.8	12.7	4.8	16.0	12.6	6.4	92.0	94.0
MMB 2201	hair	0.9981	0.005	0.025	13.1	11.4	4.3	15.0	14.3	6.2	15.5	13.1	6.5	89.8	91.2
	nails	0.9981	5	20	13.9	10.0	5.2	12.8	12.6	4.7	15.9	12.8	6.4	93.4	93.4
Pravadoline	hair	0.9976	0.005	0.025	13.6	10.7	4.3	15.4	14.1	6.6	15.9	13.0	6.7	91.4	91.2
	nails	0.9982	5	20	14.3	9.3	4.8	13.0	12.8	5.1	16.0	12.9	6.3	95.6	94.1
APP FUBINACA	hair	0.9971	0.005	0.025	13.2	10.3	4.9	15.2	14.0	6.4	15.6	12.8	6.8	89.9	85.6
	nails	0.9993	5	20	14.0	9.1	4.7	13.2	12.7	4.5	16.1	12.6	6.5	92.3	90.1
5-F ADB	hair	0.9982	0.005	0.025	12.8	10.5	4.5	15.4	13.9	6.5	15.7	12.7	7.1	90.1	87.5
	nails	0.9995	5	20	13.0	9.3	5.1	12.9	12.9	4.8	16.0	12.4	6.3	92.1	78.4
AM-2233	hair	0.9962	0.005	0.025	13.5	9.8	4.1	15.3	13.8	6.5	15.6	12.9	7.1	91.2	89.6
	nails	0.9981	5	20	14.2	10.0	4.8	13.0	12.7	4.7	15.9	11.9	6.6	93.6	91.2

CUMYL 5F PINACA	hair	0.9981	0.005	0.025	13.7	9.5	4.3	15.6	14.0	6.3	15.7	13.0	6.8	89.1	90.6
	nails	0.9995	5	20	14.0	11.0	4.6	13.2	12.6	5.0	15.8	11.8	6.7	94.0	92.4
AM-694	hair	0.9971	0.005	0.025	13.4	10.7	4.5	15.5	14.1	6.4	15.6	13.1	6.7	90.5	89.1
	nails	0.9986	5	20	14.0	9.1	5.0	13.3	12.8	4.8	16.0	11.8	6.6	92.1	89.7
JWH 302	hair	0.9981	0.002	0.025	12.9	9.8	4.3	15.5	13.8	6.4	15.7	12.8	7.0	91.0	86.1
	nails	0.9992	2	20	13.5	9.1	4.9	12.9	12.7	4.7	15.9	11.8	6.5	93.2	88.5
CUMYL PEGACLONE	hair	0.9972	0.005	0.025	14.1	9.6	4.7	15.3	13.9	6.6	15.4	12.7	7.1	89.1	85.4
	nails	0.9981	5	20	14.5	8.6	5.1	12.7	12.8	4.8	16.0	11.7	6.6	93.8	87.3
RCS-4	hair	0.9981	0.005	0.025	13.2	10.4	4.5	15.4	14.0	6.3	15.4	12.9	7.1	90.1	76.3
	nails	0.9991	5	20	14.2	9.8	5.1	13.2	12.7	4.6	16.1	11.9	6.4	93.1	87.5
JWH-251	hair	0.9991	0.003	0.025	13.7	10.8	4.6	15.1	14.0	6.5	16.0	12.8	6.9	90.6	88.2
	nails	0.9991	3	20	13.5	11.0	5.3	12.9	12.5	4.9	16.2	12.0	6.6	93.4	92.3
AM-2201	hair	0.9971	0.001	0.010	13.5	9.8	4.1	15.6	14.2	6.4	15.4	12.7	6.9	89.9	85.4
	nails	0.9989	1	10	14.2	10.0	4.8	13.2	12.9	5.0	15.9	12.0	6.4	91.2	86.8
UR 144	hair	0.9969	0.001	0.010	13.2	10.7	4.8	15.1	14.1	6.6	15.6	13.0	7.1	92.0	87.6
	nails	0.9978	1	12	14.0	10.3	4.5	13.0	12.9	4.8	15.8	11.9	6.7	95.6	89.0
JWH-203	hair	0.9962	0.003	0.025	13.7	10.8	4.6	15.4	13.8	6.2	15.9	12.8	6.9	89.0	90.0
	nails	0.9973	3	20	13.5	11.0	5.3	13.2	12.5	4.6	15.9	12.0	6.5	92.0	92.1
5F NNEI-2	hair	0.9976	0.005	0.030	13.5	9.2	3.8	15.5	13.9	6.5	15.9	12.8	7.0	91.3	90.0
	nails	0.9981	5	20	14.5	10.6	4.5	13.0	12.6	4.5	16.0	11.8	6.7	94.2	95.0
5F-AKB48	hair	0.9973	0.005	0.030	13.2	9.8	4.3	15.3	14.1	6.3	15.8	12.9	6.9	90.6	89.0
	nails	0.9981	5	20	14.0	10.5	4.9	13.2	12.7	4.7	16.1	12.0	6.5	91.3	91.4
RCS-8	hair	0.9981	0.005	0.030	13.2	10.4	4.6	15.0	14.0	6.4	15.7	12.8	7.1	89.9	86.7
	nails	0.9984	5	20	14.0	9.8	5.1	13.1	12.7	4.6	16.2	11.9	6.6	93.2	87.9
JWH-018	hair	0.9981	0.003	0.025	13.7	10.8	4.6	15.7	13.8	6.4	15.6	12.9	7.0	87.2	86.5
	nails	0.9983	3	25	13.5	11.0	5.3	13.4	12.8	4.5	16.0	12.0	6.4	90.6	88.0
CP47, 497-C8	hair	0.9989	0.005	0.030	13.4	10.4	4.2	15.6	13.7	6.5	15.4	13.0	7.0	89.1	87.6
	nails	0.9986	5	20	14.1	9.9	4.8	13.2	12.6	4.9	15.9	12.0	6.5	91.2	91.4
JWH-016	hair	0.9998	0.003	0.025	13.5	10.7	4.5	15.1	14.0	6.6	15.5	12.9	6.9	90.4	88.2
	nails	0.9993	3	20	14.0	10.5	4.9	13.5	12.8	4.9	15.9	12.1	6.6	94.1	91.2
JWH-098	hair	0.9972	0.003	0.025	13.6	10.5	4.2	15.6	14.1	6.4	15.9	12.8	6.8	88.9	88.2
	nails	0.9978	3	20	13.8	9.5	4.5	12.6	12.7	5.0	16.1	12.4	6.6	92.3	91.3

THJ 018	hair	0.9981	0.003	0.025	13.4	10.7	4.5	15.6	14.2	6.3	16.0	12.7	7.0	89.0	88.5
	nails	0.9992	3	30	14.0	9.1	5.0	12.9	12.9	4.8	15.9	12.2	6.4	92.1	91.0
JWH-081	hair	0.9971	0.003	0.025	12.8	9.8	4.0	15.4	13.8	6.4	15.8	12.8	7.1	90.4	88.0
	nails	0.9976	3	20	13.6	8.7	4.5	12.8	12.5	4.7	16.0	12.1	6.6	91.2	90.8
JWH-122	hair	0.9984	0.003	0.020	13.7	10.5	4.5	15.2	13.9	6.3	15.8	12.9	6.9	89.7	87.8
	nails	0.9986	3	15	13.9	10.0	5.1	13.0	13.0	4.8	16.2	12.0	6.8	93.4	91.6
JWH-019	hair	0.9988	0.003	0.025	13.2	10.6	4.3	15.7	14.0	6.6	15.7	13.0	6.8	90.1	93.5
	nails	0.9991	3	20	14.0	10.4	4.8	13.0	12.7	4.9	16.2	12.0	6.7	93.2	95.4
JWH-007	hair	0.9979	0.003	0.025	13.4	10.7	4.5	15.4	13.8	6.5	15.6	12.9	7.0	89.4	91.6
	nails	0.9984	3	20	14.1	10.4	4.9	12.7	12.6	4.8	16.1	12.4	6.4	91.2	94.6
JWH-210	hair	0.9989	0.003	0.025	13.5	10.2	4.6	15.3	13.7	6.5	15.7	12.7	7.1	90.3	92.8
	nails	0.9989	3	20	14.0	9.8	5.1	12.8	12.6	4.7	16.1	11.7	6.9	91.2	96.0
JWH-147	hair	0.9971	0.003	0.025	13.6	9.8	4.3	15.3	13.7	6.4	15.6	12.6	6.9	89.7	92.6
	nails	0.9975	3	20	13.4	9.6	4.9	12.7	12.6	4.8	16.0	12.0	6.6	92.1	96.0
JWH-398	hair	0.9988	0.003	0.025	13.2	11.0	4.5	15.5	14.3	6.4	15.7	12.5	7.1	90.1	93.4
	nails	0.9991	3	20	14.0	10.2	5.0	13.0	12.8	4.8	16.0	12.1	6.5	90.3	96.4
CB-13	hair	0.9971	0.005	0.030	14.6	10.6	4.8	15.4	14.3	6.3	15.6	12.9	7.0	87.9	95.1
	nails	0.9976	5	20	14.8	10.7	5.3	12.6	12.5	4.9	16.0	12.1	6.5	90.1	97.0
fentanyl analogues															
Methoxyacetyl norfentanyl	hair	0.9972	0.001	0.002	11.8	12.3	7.4	11.9	9.7	5.4	12.6	10.0	9.1	90.5	80.3
	nails	0.9997	0.5	1	12.3	12.3	10.4	12.7	10.7	11.4	14.0	7.0	9.1	90.2	80.3
Acetyl Norfentanyl	hair	0.9984	0.001	0.002	13.1	8.1	7.5	12.8	7.0	7.4	12.3	5.8	9.5	92.3	91.3
	nails	0.9990	0.5	1	13.2	9.1	7.5	11.2	6.0	6.4	12.3	10.8	9.5	95.3	92.0
Butyryl Fentanyl Carboxy metabolite	hair	0.9976	0.001	0.002	12.5	12.8	7.4	12.2	9.4	10.9	12.9	9.1	9.2	90.8	86.9
	nails	0.9976	0.5	1	11.4	10.8	11.4	11.7	12.4	11.9	11.3	7.1	8.2	90.7	86.9
Valeryl Fentanyl Carboxy metabolite	hair	0.9968	0.001	0.002	12.7	9.2	10.3	13.2	7.2	8.6	11.2	9.3	11.5	90.8	93.3
	nails	0.9984	0.5	1	13.2	11.2	8.3	11.2	11.2	9.6	11.6	8.3	7.5	97.3	90.3
Methoxyacetylfentanyl	hair	0.9972	0.001	0.002	11.8	12.3	7.4	11.9	9.7	5.4	12.6	10.0	9.1	90.5	80.3
	nails	0.9971	0.5	1	11.7	12.3	7.4	11.3	9.7	5.4	12.2	10.0	9.1	90.3	80.3
Furanyl Norfentanyl	hair	0.9921	0.001	0.002	12.6	8.5	5.0	14.2	11.0	3.5	11.5	13.2	9.8	90.4	95.2
	nails	0.9982	0.5	1	11.9	9.5	7.0	12.4	11.0	8.5	13.9	14.2	9.8	90.7	93.2
	hair	0.9987	0.001	0.002	13.1	8.1	7.5	12.8	7.0	7.4	12.3	5.8	9.5	90.3	85.3

Compound	Matrix													
Cis-3-metyl Norfentanyl	nails	0.9985	0.5	1	10.2	8.5	4.8	10.9	11.1	8.8	12.1	10.9	8.1	90.1	89.4
Trans-3-metyl Norfentanyl	hair	0.9987	0.001	0.002	13.1	8.1	7.5	12.8	7.0	7.4	12.3	5.8	9.5	90.3	85.3
	nails	0.9985	0.5	1	10.2	8.5	4.8	10.9	11.1	8.8	12.1	10.9	8.1	90.1	89.4
Butyryl Norfentanyl	hair	0.9976	0.001	0.002	12.6	5.5	3.8	10.4	8.1	9.8	10.7	8.9	10.1	91.1	90.7
	nails	0.9978	0.5	1	10.2	8.5	4.8	10.9	11.1	8.8	12.1	10.9	8.1	91.3	88.7
Cyclopropyl norfentanyl	hair	0.9989	0.001	0.002	10.1	5.2	8.8	10.4	10.0	7.9	12.3	9.4	8.7	90.8	95.7
	nails	0.9986	0.5	1	10.2	5.2	8.8	11.0	10.0	7.9	12.8	9.4	8.7	90.4	95.7
β-hydroxyfentanyl	hair	0.9987	0.001	0.002	11.2	11.9	10.1	12.1	11.2	13.6	11.9	8.9	7.5	90.4	88.5
	nails	0.9991	0.5	1	10.4	7.9	8.1	12.3	8.2	11.6	10.9	8.9	7.5	90.6	88.5
Alfentanyl	hair	0.9983	0.001	0.002	10.5	7.6	9.5	10.9	7.2	10.8	11.4	10.4	11.5	90.7	88.3
	nails	0.9983	0.5	1	10.1	7.6	7.5	11.8	11.2	9.8	10.9	11.4	9.5	90.2	80.3
Cyclopropylfentanyl	hair	0.9978	0.001	0.002	13.6	9.2	10.2	12.1	7.3	8.6	12.8	9.3	11.5	91.3	93.3
	nails	0.9979	0.5	1	12.4	9.2	10.2	12.2	7.3	8.6	12.4	9.3	11.5	91.1	93.3
β-hydroxythiofentanyl	hair	0.9988	0.001	0.002	13.6	13.1	10.2	12.3	9.4	8.3	12.7	10.2	11.7	90.6	90.2
	nails	0.9989	0.5	1	11.2	12.1	7.2	13.1	11.4	7.3	11.7	5.2	5.7	90.1	85.2
Furanylethyl Fentanyl	hair	0.9997	0.001	0.002	11.8	5.9	7.7	14.0	10.5	9.4	11.7	11.5	8.5	90.3	90.9
	nails	0.9996	0.5	1	12.1	5.9	6.7	12.3	10.5	11.4	10.4	8.5	7.5	90.3	89.9
Acetyl fentanyl	hair	0.9989	0.001	0.002	12.2	9.6	9.7	12.9	11.2	10.7	11.9	9.2	9.6	91.1	85.2
	nails	0.9989	0.5	1	10.2	11.6	10.7	9.9	8.0	10.7	12.9	9.2	7.6	90.1	86.2
Furanyl Fentanyl	hair	0.9976	0.001	0.002	10.7	8.7	8.2	13.5	10.8	9.6	12.5	9.3	10.2	91.5	74.7
	nails	0.9976	0.5	1	10.8	8.7	8.2	11.4	10.6	9.8	12.8	10.3	10.2	90.5	74.9
Carfentanyl	hair	0.9978	0.001	0.002	13.0	8.3	5.9	11.7	11.5	10.9	12.2	8.2	10.9	90.5	90.3
	nails	0.9980	0.5	1	10.9	11.3	8.9	11.7	10.5	7.9	12.2	10.2	9.9	90.2	90.3
Butyryl Fentanyl	hair	0.9989	0.001	0.002	12.6	5.5	3.8	10.4	8.1	9.8	10.7	8.9	10.1	91.1	90.7
	nails	0.9991	0.5	1	10.7	7.8	9.2	11.8	8.8	8.9	11.0	11.1	13.8	90.2	90.6
Despropionyl-para-fluorofentanyl	hair	0.9978	0.001	0.002	11.5	8.3	6.8	12.3	4.4	8.0	12.9	9.0	73.0	91.7	87.6
	nails	0.9982	0.5	1	11.8	9.8	7.8	10.5	4.4	6.0	12.3	3.0	4.3	91.2	90.6
4-ANPP	hair	0.9988	0.001	0.002	10.6	7.7	7.2	11.5	9.8	9.3	13.4	9.5	10.9	91.2	70.7
	nails	0.9998	0.5	1	10.7	7.7	5.2	11.9	9.8	11.3	10.6	7.5	4.9	90.2	91.7
Phenyl Acetyl Fentanyl	hair	0.9976	0.001	0.002	10.1	7.2	6.8	11.9	9.0	7.9	11.9	9.4	7.7	91.1	90.7
	nails	0.9978	0.5	1	10.3	8.2	9.8	12.6	7.0	11.9	10.9	8.4	9.7	90.4	91.7

synthetic cathinones														
4-fluoromethcathinone	hair	0.9993	0.002	0.020	12.1	6.6	5.0	9.1	5.0	3.0	17.4	4.3	3.0	88.4	73.4
	nails	0.9997	2	10	13.0	8.0	7.0	14.1	10.2	8.0	18.1	6.0	4.0	91.2	76.9
Methcathinone	hair	0.9992	0.002	0.020	12.3	6.4	5.0	9.2	5.5	3.1	17.3	4.2	2.8	89.1	80.1
	nails	0.9997	2	10	12.8	7.8	6.8	13.8	10.2	7.9	17.9	6.1	4.3	90.1	86.4
Methylone	hair	0.9981	0.002	0.020	12.0	6.7	4.8	9.1	5.1	3.0	17.3	4.2	2.8	88.7	84.5
	nails	0.9989	2	10	12.9	8.1	6.5	13.8	10.2	7.8	18.0	6.0	4.3	91.2	83.1
Mephedrone	hair	0.9978	0.002	0.020	11.9	6.8	5.1	9.5	5.1	2.9	17.5	4.4	2.7	90.1	91.0
	nails	0.9981	2	10	12.5	8.2	6.9	14.1	10.1	7.9	17.9	6.2	4.0	93.2	93.2
Buphedrone	hair	0.9993	0.002	0.020	11.6	6.4	4.8	9.3	4.7	2.8	17.3	4.6	2.9	90.2	87.4
	nails	0.9993	2	10	12.7	7.6	7.0	14.2	10.1	7.7	18.0	6.0	4.0	92.1	90.3
Ethylone	hair	0.9985	0.002	0.020	12.6	6.4	5.6	8.7	5.0	2.9	17.5	4.7	3.1	89.9	90.2
	nails	0.9991	2	10	13.5	7.8	7.5	13.9	9.9	8.0	18.1	6.0	4.0	91.2	92.1
Buthylone	hair	0.9976	0.002	0.020	12.3	6.3	4.5	8.9	4.7	3.0	17.4	4.5	3.1	90.1	89.2
	nails	0.9989	2	10	13.2	7.8	6.8	14.3	10.2	8.0	18.2	6.1	4.1	92.4	93.2
Dimethylcathinone	hair	0.9983	0.002	0.020	11.8	6.6	4.7	8.8	5.0	3.1	17.2	4.0	3.2	89.9	78.1
	nails	0.9985	2	10	12.9	7.6	6.5	14.1	10.1	8.1	17.9	6.1	3.9	93.2	78.3
Diethylcathinone	hair	0.9988	0.002	0.020	11.7	6.7	5.2	9.1	4.9	3.2	17.6	4.3	2.9	90.6	81.4
	nails	0.9991	2	10	12.5	7.9	7.1	13.7	9.8	8.3	18.1	6.2	3.8	92.1	85.6
3,4-dimethylmethcathinone	hair	0.9986	0.002	0.020	12.1	6.6	5.0	9.2	4.9	2.8	17.4	4.1	2.8	90.3	90.1
	nails	0.9991	2	10	13.0	8.0	7.0	14.4	9.9	8.1	18.2	6.0	3.8	92.3	92.4
4-methylethcathinone	hair	0.9996	0.002	0.020	12.1	6.6	5.0	8.8	5.2	2.9	17.4	4.1	2.9	90.3	75.4
	nails	0.9996	2	10	13.0	8.0	7.0	13.9	10.0	8.0	18.1	5.9	4.0	91.2	73.1
Ethcathinone	hair	0.9983	0.002	0.020	12.4	6.7	5.2	9.0	5.2	3.1	17.5	4.1	3.0	89.9	87.5
	nails	0.9987	2	10	13.2	8.2	7.2	14.1	10.2	7.8	17.9	5.9	4.1	92.6	83.1
Penthedrone	hair	0.9994	0.002	0.020	11.9	6.6	4.5	9.0	5.0	3.0	17.6	4.5	2.9	91.0	83.3
	nails	0.9997	2	10	12.5	7.5	7.0	14.1	10.1	8.1	18.0	5.8	4.2	94.3	83.9
Penthylone	hair	0.9983	0.002	0.020	12.2	6.4	4.9	9.1	5.1	2.8	17.4	4.2	2.7	90.8	86.1
	nails	0.9984	2	10	13.7	6.8	6.5	13.9	10.2	8.2	18.1	5.7	4.1	94.3	88.3
MDPV	hair	0.9976	0.002	0.020	11.7	6.7	5.3	9.2	4.9	3.1	17.5	4.3	2.9	91.8	90.0
	nails	0.9984	2	10	12.5	7.7	6.9	14.0	10.0	8.0	18.1	6.0	4.0	92.0	87.2

Naphyrone	hair	0.9971	0.002	0.020	12.6	6.4	5.6	9.2	5.1	3.2	17.3	4.1	3.0	90.3	85.6
	nails	0.9981	2	10	13.5	7.8	7.5	14.0	9.9	8.0	18.2	5.9	4.0	90.4	86.3
Tryptamine															
AcO DMT	hair	0.9984	0.002	0.006	14.0	15.1	8.1	14.8	15.0	7.7	14.6	13.8	8.6	90.1	85.6
	nails	0.9987	1	6	13	12.9	9.0	12,9	14.1	10.4	16.0	12.1	8.4	90.4	87.9
5-MeO AMT	hair	0.9984	0.002	0.006	14.4	15.8	8.3	14.5	15,2	7.6	14.4	13.9	8.5	89.9	90.1
	nails	0.9987	1	6	13.2	12.8	9.1	12.9	14.1	10.3	15.9	12.0	8.6	91.2	94.3
4-OH DET	hair	0.9987	0.002	0.006	13.8	15.5	8.4	14.6	15.2	7.6	14.6	13.9	8.4	90.7	81.2
	nails	0.9993	1	6	12.5	12.6	8.7	13.0	13.8	10.3	15.8	11.9	8.6	91.0	78.3
5-MeO MIPT	hair	0.9978	0.002	0.006	13.8	14.9	7.9	14.8	15.0	7.5	14.5	14.1	8.7	90.6	86.6
	nails	0.9989	1	6	12.7	12.5	9.2	12.8	14.1	10.2	16.2	11.8	8.5	92.0	88.1
4-AcO DIPT	hair	0.9981	0.002	0.006	14.2	14.5	8.1	14.5	14.9	7.8	14.7	13.8	8.3	91.2	79.7
	nails	0.9989	1	6	13.0	13.2	9.0	12.9	13.8	10.4	16.1	11.8	8.6	91.5	77.5
5-MeO DPT	hair	0.9978	0.002	0.006	13.5	15.0	7.5	14.7	14.9	7.8	14.5	13.8	8.5	90.6	85.4
	nails	0.9983	1	6	13.6	13.1	9.2	13.1	13.8	10.4	15.9	12.4	8.4	92.0	86.1
5-MeO DALT	hair	0.9991	0.002	0.006	14.8	14.7	8.1	14.8	14.9	7.9	14.5	13.7	8.2	90.1	86.8
	nails	0.9994	1	6	13.4	12.7	8.9	12.5	14.5	10.2	15.9	12.1	8.5	90.9	86.2
Phenylethylamines															
Ritalinic acid	hair	0.9976	0.005	0.020	16.5	7.7	2.5	9.1	5.0	3.0	17.4	4.3	3.0	92.0	83.2
	nails	0.9979	5	18	15.8	7.1	2.2	14.1	10.2	8.0	18.1	6.0	4.0	92.5	84.1
5 APB	hair	0.9961	0.005	0.020	16.4	7.8	2.6	9.3	5.1	3.3	17.6	4.3	2.9	91.2	86.8
	nails	0.9977	5	18	15.9	7.1	2.6	14.1	10.2	8.0	17.9	5.9	3.8	92.0	89.6
6 APB	hair	0.9985	0.005	0.020	16.7	7.5	2.2	9.1	5.1	3.0	17.3	4.6	3.0	89.9	91.0
	nails	0.9989	5	18	15.6	6.8	1.8	14.0	10.1	7.9	18.1	5.8	4.3	90.7	87.4
6 MAPB	hair	0.9967	0.005	0.020	16.8	7.6	2.4	9.1	4.8	2.9	17.6	4.2	2.9	90.1	85.4
	nails	0.9975	5	18	16.0	6.9	2.2	14.0	10.2	7.7	18.2	5.9	3.6	90.8	87.1
5 EAPB	hair	0.9987	0.005	0.020	16.1	7.4	2.5	9.0	4.9	2.9	17.6	4.2	2.8	91.2	78.6
	nails	0.9991	5	18	15.8	7.5	2.4	14.3	9.8	7.8	18.1	6.1	4.1	92.0	76.1
MPHP	hair	0.9983	0.005	0.020	16.5	7.7	2.5	8.9	5.0	2.9	17.1	4.0	2.9	90.5	88.1
	nails	0.9990	5	18	15.6	7.2	2.1	14.2	9.9	8.3	17.8	5.8	4.1	91.2	84.4

MDA: 3,4-Methylenedioxyamphetamine; MDMA: 3,4-Methylenedioxymetamphetamine; 6-O-MAM: 6-O-Monoacetylmorphine; MDEA: 3,4-methylenedioxy-N-ethylamphetamine; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; THC: $\Delta 9$-tetrahydrocannabinol; 4-ANPP: 4-anilino-N-phenethyl-piperidine; MDPV:

Methylenedioxypyrovalerone; AcO DMT: Acetyl-o-dimethyltryptamine; 5-MeO-AMT: 5-methoxy-α-methyltryptamine; 4-OH DET: 4-hydroxy-diethyltryptamine; 5-MeO-MIPT: 5-methoxy-N-methyl-N-isopropyltryptamine; 4-AcO-DIPT: 4-acetoxy-diisopropyltryptamine; 5-MeO-DPT: 5-methoxy-dipropyltryptamine; 5-MeO-DALT: 5-methoxy-diallyltryptamine; 5-APB: 5-(2-aminopropyl)benzofuran; 6-APB: 6-(2-aminopropyl)benzofuran; 6-MAPB: 1-(benzofuran-6-yl)-N-methylpropan-2-amine; 5-EAPB: 1-(benzofuran-5-yl)-N-ethylpropan-2-amine; MPHP: 4'-methyl-α-pyrrolidinohexiophenone;

Table 3. Target analytes detected in real hair samples (ng/mg)

Sample	Amphetamine	MDMA	Ketamine	Norketamine	BZG	Cocaine	Morphine	Codeine	6-O-MAM	Methadone	EDDP	Tramadol	THC	5 MeO AMT	6 APB	UR 144	APP FUBINACA	Mephedrone	Fentanyl	Norfentanyl	4-ANPP	Butyryl Fentanyl	Butyryl Norfentanyl
A	0.1	-	-	-	9.0	41.2	-	-	-	5.3	0.2	15.0	-	-	-	-	-	-	3.2	0.16	0.007	-	-
B	0.1	-	-	-	7.7	36.6	-	-	-	4.6	0.2	12.3	-	-	-	-	-	-	2.8	0.14	0.007	-	-
C	-	-	-	-	0.1	0.1	-	-	-	6.2	0.4	-	1.7	-	-	0.10	-	-	-	-	-	-	-
D	-	-	-	-	-	-	-	-	-	38.6	1.8	-	-	0.07	-	-	-	-	-	-	-	-	-
E	-	0.1	0.08	0.05	0.12	1.9	2.1	0.6	1.3	9.8	0.5	-	2.3	-	-	-	-	-	-	-	-	0.38	0.16
F	-	-	-	-	-	-	-	0.4	-	23.4	1.2	-	-	-	-	-	0.05	-	-	-	-	-	-
G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-
H	-	0.2	0.13	0.04	-	0.3	0.9	0.2	1.4	0.3	0.1	-	0.4	-	-	-	-	0.24	-	-	-	-	-
J	-	5.5	0.9	0.1	2.1	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	-	77.2	27.3	8.4	1.9	67.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MDMA: 3,4-Methylenedioxymethamphetamine; BZG: benzoylecgonine; 6-O-MAM: 6-O-monoacetylmorphine; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; THC: Δ9-tetrahydrocannabinol; 5-MeO AMT: 5-methoxy-α-methyltryptamine; 6-APB: 6-(2-aminopropyl)benzofuran; 4-ANPP: 4anilino-N-phenethyl-piperidine.

Table 4. Target analytes detected in real nail samples (pg/mg)

Sample	MDA	MDMA	Ketamine	Norketamine	BZG	Cocaine	Buphedrone	Mephedrone	Lorazepam	Methamphetamine	JWH-081	JWH-122	JWH-398	THC
L	60	3000	860	210	1886	9724	-	-	1	1	-	-	-	5
M	359	7000	240	580	1204	1181	2	-	-	1	-	-	-	3
N	3	1	170	4	4968	14344	1	101	170	710	-	-	-	-
O	10	6	1307	8	14278	19818	3	136	220	236	-	-	-	-
P	31	29000	970	113	19044	81475	-	410	-	439	-	-	-	1
Q	67	1000	284	510	550	200	-	1	-	390	-	-	-	4
R	-	-	-	-	129	322	-	-	-	-	24	31	64	17
S	-	-	321	123	67	143	-	-	-	-	17	-	71	4
T	-	-	65	13	-	-	-	-	-	-	35	22	-	6

MDA: 3,4-Methylenedioxyamphetamine; MDMA: 3,4-Methylendioxymethamphetamine; BZG: benzoylecgonine; THC: Δ9-tetrahydrocannabinol